GLYCOSPHINGOLIPIDS

OF HUMAN MECONIUM

A rediscovered source of

fetal and blood group antigens.

Göran Larson

Göteborg 1982

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41544702_0004

GLYCOSPHINGOLIPIDS

OF HUMAN MECONIUM

A rediscovered source of

fetal and blood group antigens.

AKADEMISK AVHANDLING
som, för avläggande av doktorsexamen i
medicinsk vetenskap vid Göteborgs Universitet,
offentligen kommer att försvaras i Föreläsningssalen,
Institutionen för Medicinsk och Fysiologisk Kemi,
Göteborgs Universitet
kl 09.15 fredagen den 3 december 1982

av
Göran Larson

Göteborg 1982

ABSTRACT

Larson, G. Glycosphingolipids of human meconium, a rediscovered source of fetal and blood group antigens. pp. 1 - 36. Department of Medical Biochemistry, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

This thesis is based on the following publications:

I. Karlsson, K.-A. and Larson, G. Molecular characterization of cell-surface antigens of human fetal tissue. Meconium, a rich source of epithelial blood-group glycolipids. (1978) FEBS Lett. 87, 283-287.

II. Karlsson, K.-A. and Larson, G. Structural characterization of lactotetraosylceramide, a novel glycosphingolipid isolated from human meconium. (1979) J. Biol. Chem. 254, 9311-9316.

III. Karlsson, K.-A. and Larson, G. Molecular characterization of cell-surface antigens of fetal tissue. Detailed analysis of glycosphingolipids of meconium of a human O Le(a-b+) secretor. (1981) J. Biol. Chem. 256, 3512-3524.

IV. Karlsson, K.-A. and Larson, G. Potential use of glycosphingolipids of human meconium for blood group chemotyping of single individuals. (1981) FEBS Lett. 128, 71-74.

Glycosphingolipids comprise a group of membrane components, of great structural variability, found mainly at the surface of cells. As blood group antigens, differentiation antigens, tumor antigens and as receptors for various ligands glycosphingolipids have been associated with several biological phenomena such as immune recognition, cellular differentiation, oncogenic transformation and host-pathogen interactions.
The present work introduces meconium, the first sterile stools of newborns, as an easily available rich source of fetal and blood group antigens and as a model for structural studies on glycosphingolipids during intestinal differentiation. Non-acid and acid glycosphingolipids were prepared from several individual meconia, freed from non-glycolipid contaminants and chemically characterized using thin-layer chromatography, gas-liquid chromatography, mass spectrometry and NMR spectroscopy of permethylated and $LiAlH_4$-reduced permethylated glycolipids. Immunological characterizations were initially done as hemagglutination-inhibition assays but has now been improved to radioimmunoassays and a technique of binding antibodies directly to thin-layer chromatograms.
Meconium was shown to be rich in glycolipids, especially blood group active fucolipids, and the various patterns of glycolipids found in individual meconia were shown to correlate to the ABH and Lewis blood groups and to the secretor status of the children. Using the above techniques of structural characterization glycosphingolipids of human meconia were used for a chemical typing of single individuals with a comparable immunological typing on blood and saliva. Patterns, not encountered in adult intestines, of co-presence of probable precursors and products were characteristic for non-acid glycolipids of meconium. Thus, lactotetraosylceramide (Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer), a blood group H type 1 chain pentaglycosylceramide (Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer), a Lewis a active pentaglycosylceramide (Galβ1→3GlcNAc(4←1αFuc)β1→3Galβ1→4Glcβ1→1Cer) and a Lewis b active hexaglycosylceramide (Fucα1→2Galβ1→3GlcNAc(4←1αFuc)β1→3Galβ1→4Glcβ1→1Cer) with identical ceramides (mainly trihydroxy 18:0 long chain bases and 2-hydroxy 16:0 and 20:0-24:0 fatty acids) were all isolated and characterized from one single case. Free ceramide, galactosyl-, glucosyl-, lactosyl-, digalactosyl-, globotriaosyl- and globotetraosylceramides were also characterized from this meconium as well as a novel glycolipid having a blood group H determinant based on a globopentaose oligosaccharide, a hitherto unknown linkage between the human ABH and P blood group systems. Minor novel glycolipids having 0-3 fucoses in their carbohydrate chains and carrying up to 11 sugar residues were also identified. Among the acid glycolipids sulfatides, with 1 or 2 sugar residues, and hematoside (sialyllactosylceramide) were the altogether dominating species although also more complex gangliosides were detected.
The biological significance of meconium is discussed in light of the recent findings of immunological cross-reactivities between glycolipids of human meconium and gastrointestinal tumors as well as the possible protective role for glycolipids of meconium at the time of bacterial colonization of the intestines.

Key words: Human meconium, intestinal differentiation, glycosphingolipids, blood group antigens, fetal antigens, mass spectrometry, NMR spectroscopy.

ISBN 91-7222-531-9

Department of Medical Biochemistry
University of Göteborg
Göteborg, Sweden

GLYCOSPHINGOLIPIDS OF HUMAN MECONIUM

A rediscovered source of fetal and blood group antigens.

Göran Larson

Göteborg 1982

ABSTRACT

Larson, G. Glycosphingolipids of human meconium, a rediscovered source of fetal and blood group antigens. pp. 1 - 36. Department of Medical Biochemistry, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

This thesis is based on the following publications:

I. Karlsson, K.-A. and Larson, G. Molecular characterization of cell-surface antigens of human fetal tissue. Meconium, a rich source of epithelial blood-group glycolipids. (1978) FEBS Lett. 87, 283-287.

II. Karlsson, K.-A. and Larson, G. Structural characterization of lactotetraosylceramide, a novel glycosphingolipid isolated from human meconium. (1979) J. Biol. Chem. 254, 9311-9316.

III. Karlsson, K.-A. and Larson, G. Molecular characterization of cell-surface antigens of fetal tissue. Detailed analysis of glycosphingolipids of meconium of a human O Le(a-b+) secretor. (1981) J. Biol. Chem. 256, 3512-3524.

IV. Karlsson, K.-A. and Larson, G. Potential use of glycosphingolipids of human meconium for blood group chemotyping of single individuals. (1981) FEBS Lett. 128, 71-74.

Glycosphingolipids comprise a group of membrane components, of great structural variability, found mainly at the surface of cells. As blood group antigens, differentiation antigens, tumor antigens and as receptors for various ligands glycosphingolipids have been associated with several biological phenomena such as immune recognition, cellular differentiation, oncogenic transformation and host-pathogen interactions.
The present work introduces meconium, the first sterile stools of newborns, as an easily available rich source of fetal and blood group antigens and as a model for structural studies on glycosphingolipids during intestinal differentiation. Non-acid and acid glycosphingolipids were prepared from several individual meconia, freed from non-glycolipid contaminants and chemically characterized using thin-layer chromatography, gas-liquid chromatography, mass spectrometry and NMR spectroscopy of permethylated and $LiAlH_4$-reduced permethylated glycolipids. Immunological characterizations were initially done as hemagglutination-inhibition assays but has now been improved to radioimmunoassays and a technique of binding antibodies directly to thin-layer chromatograms.
Meconium was shown to be rich in glycolipids, especially blood group active fucolipids, and the various patterns of glycolipids found in individual meconia were shown to correlate to the ABH and Lewis blood groups and to the secretor status of the children. Using the above techniques of structural characterization glycosphingolipids of human meconia were used for a chemical typing of single individuals with a comparable immunological typing on blood and saliva. Patterns, not encountered in adult intestines, of co-presence of probable precursors and products were characteristic for non-acid glycolipids of meconium. Thus, lactotetraosylceramide (Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer), a blood group H type 1 chain pentaglycosylceramide (Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer), a Lewis a active pentaglycosylceramide (Galβ1→3GlcNAc(4←1αFuc)β1→3Galβ1→4Glcβ1→1Cer) and a Lewis b active hexaglycosylceramide (Fucα1→2Galβ1→3GlcNAc(4←1αFuc)β1→3Galβ1→4Glcβ1→1Cer) with identical ceramides (mainly trihydroxy 18:0 long chain bases and 2-hydroxy 16:0 and 20:0-24:0 fatty acids) were all isolated and characterized from one single case. Free ceramide, galactosyl-, glucosyl-, lactosyl-, digalactosyl-, globotriaosyl- and globotetraosylceramides were also characterized from this meconium as well as a novel glycolipid having a blood group H determinant based on a globopentaose oligosaccharide, a hitherto unknown linkage between the human ABH and P blood group systems. Minor novel glycolipids having 0-3 fucoses in their carbohydrate chains and carrying up to 11 sugar residues were also identified. Among the acid glycolipids sulfatides, with 1 or 2 sugar residues, and hematoside (sialyllactosylceramide) were the altogether dominating species although also more complex gangliosides were detected.
The biological significance of meconium is discussed in light of the recent findings of immunological cross-reactivities between glycolipids of human meconium and gastrointestinal tumors as well as the possible protective role for glycolipids of meconium at the time of bacterial colonization of the intestines.

Key words: Human meconium, intestinal differentiation, glycosphingolipids, blood group antigens, fetal antigens, mass spectrometry, NMR spectroscopy.

ISBN 91-7222-531-9

UNIV. LEIDEN BIBL.

The present thesis is based on the following publications:

I MOLECULAR CHARACTERIZATION OF CELL-SURFACE ANTIGENS OF HUMAN FETAL TISSUE. Meconium, a rich source of epithelial blood-group glycolipids. (1978) FEBS Lett. 87, 283-287.
Together with K.-A. Karlsson.

II STRUCTURAL CHARACTERIZATION OF LACTOTETRAOSYLCERAMIDE, A NOVEL GLYCOSPHINGOLIPID ISOLATED FROM HUMAN MECONIUM. (1979) J. Biol. Chem. 254, 9311-9316.
Together with K.-A. Karlsson.

III MOLECULAR CHARACTERIZATION OF CELL SURFACE ANTIGENS OF FETAL TISSUE. Detailed analysis of glycosphingolipids of meconium of a human O Le(a-b+) secretor. (1981) J. Biol. Chem. 256, 3512-3524.
Together with K.-A. Karlsson.

IV POTENTIAL USE OF GLYCOSPHINGOLIPIDS OF HUMAN MECONIUM FOR BLOOD GROUP CHEMOTYPING OF SINGLE INDIVIDUALS. (1981) FEBS Lett. 128, 71-74.
Together with K.-A. Karlsson.

These papers will be referred to in the text by their Roman numerals.

CONTENTS

Page

1. INTRODUCTION AND AIM OF THE STUDY..... 5

2. ON THE NATURE OF MECONIUM..... 12

3. GLYCOSPHINGOLIPID METHODOLOGY..... 14
   3.1 Preparation of glycosphingolipids..... 15
   3.2 Chemical characterization of glycosphingolipids..... 16
   3.3 Immunological characterization of glycosphingolipids 18

4. CURRENT WORK..... 19

5. FUTURE WORK..... 24

6. ACKNOWLEDGMENTS..... 28

7. REFERENCES..... 29

1. INTRODUCTION AND AIM OF THE STUDY

Glycosphingolipids are a group of amphipatic molecules generally considered to be present only in the outer leaflet of cell plasma membranes (Singer and Nicolson, 1972; Gahmberg and Hakomori, 1973; Steck and Dawson, 1974). Their exact subcellular and molecular organization in membranes is, however, unclear (Sakakibara et al., 1981; Hakomori, 1981a; Karlsson, 1982). A schematic model of a cell plasma membrane is depicted in Fig. 1, illustrating the proposed molecular arrangements of the major membrane components.

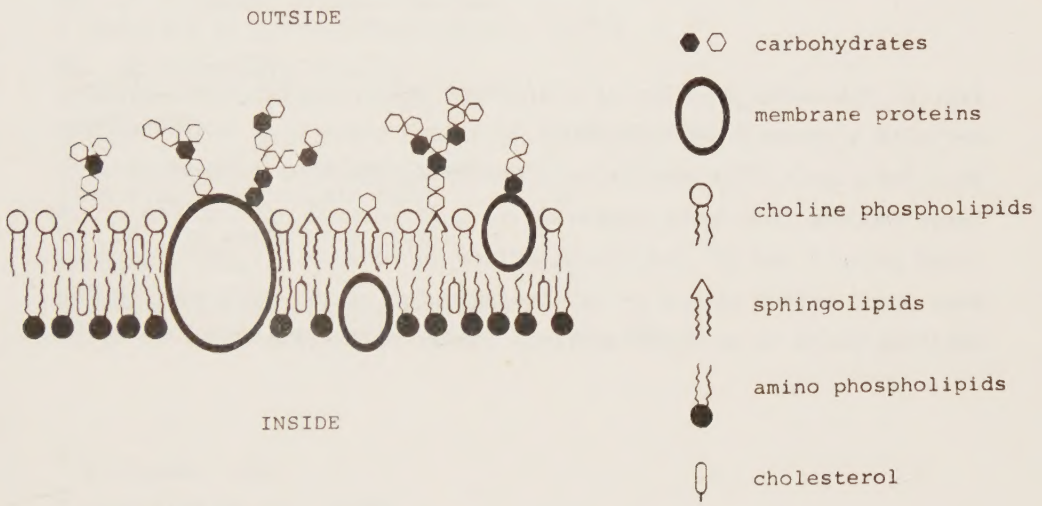

Fig. 1. Schematic model of a cell plasma membrane

Glycosphingolipids have been isolated from vertebrates, invertebrates, plants, yeast and other microorganisms and the structural diversity in this group of compounds is characteristic. Structural variability is found both in the lipophilic (ceramide) and the hydrophilic (carbohydrate) parts of the molecules (Fig. 2, Table I) and has been the subject of several recent reviews (Hakomori and Kobata, 1974; Sweeley and Siddiqui, 1977; Macher and Sweeley, 1978; McKibbin, 1978; Karlsson, 1982; Ledeen and Yu, 1982).

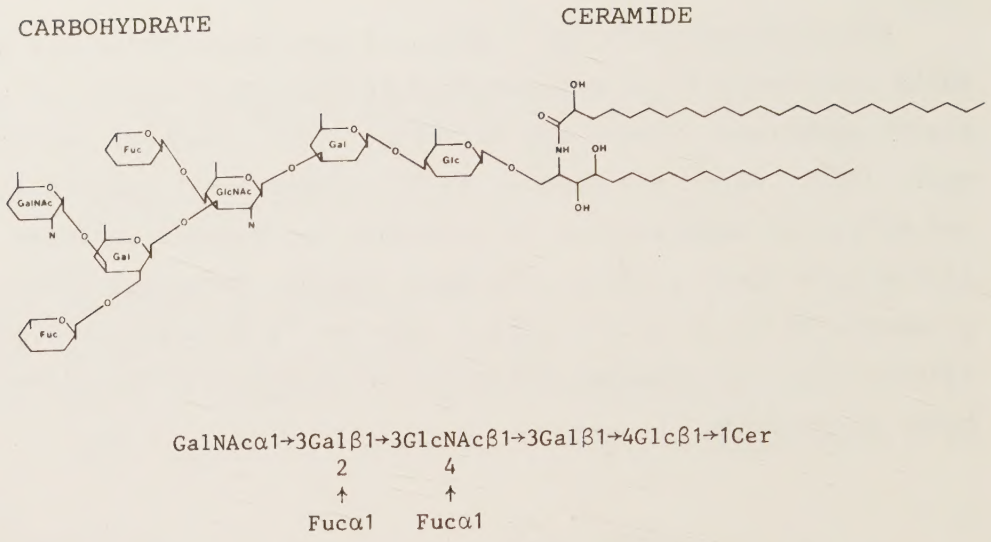

Fig. 2. Schematic formulas of a glycosphingolipid with seven sugars and with a ceramide of trihydroxy 18:0 long-chain base and 2-hydroxy 24:0 fatty acid. This particular glycolipid, possible to isolate in large amounts from human meconium, is a structural combination of the blood group A and Le^b determinants (see Table III). It does not however react either with A or Le^b antisera but reacts with the Siedler antibody which is an A_1Le^b antibody (Watkins, 1978; Brockhaus et al., 1981).

Although the total number of theoretical glycosphingolipid species would be immense when combining all the structural characteristics of Table I (disaccharides of two different hexoses gives about 70 theoretical isomers), indeed only a fraction of these species have so far been isolated and characterized (Table I). Rather, there appears to be a variation in distribution of a limited number of glycolipids between tissues and cells of different species and developmental stages. Whether this is true and possibly for cell economy reasons saving biosynthetic enzymes (see Karlsson, 1982) or simply due to limitations of the present methodology to detect more species remains to be shown.

Table I. Structural variability of glycosphingolipids

CHARACTERISTICS	SUBUNITS	
	Ceramide moiety[a]	
	Fatty acid	Long-chain base
No. of carbon atoms	14-28,35	12-25
No. of double bonds	0-2	0-2
No. of methyl branches	0-1	0-1
No. of hydroxy groups	0-2	2-3
	Carbohydrate moiety	
No. of monosaccharides in a single carbohydrate chain	1-60[b] (about)	
No. of different monosaccharides identified in glycosphingolipids	25[a,c]	
No. of glycosidic binding positions on a single monosaccharide	4-5	
No. of anomeric configurations of a single glycosidic bond	2	
No. of ring forms of a single monosaccharide	2	
No. of different oligosaccharides identified in glycosphingolipids	150 (about)	

[a] Karlsson, 1982

[b] Kościelak et al., 1976

[c] including two mono-acetyl, four mono-sulfate and six mono-methyl monosaccharides.

No physiological function has yet been proven for any particular glycolipid although several biological phenomena have been linked to glycosphingolipids (Table II). Possible functional interrelationships between these and other structurally related membrane-bound or free glycoconjugates (i.e. glycoproteins, glycoglycerolipids or free oligosaccharides) remain largely unexplored (Kobata, 1977; Rauvala and Finne, 1979; Wilczynska et al., 1980; Slomiany et al., 1981; Zopf et al., 1979).

Table II. Postulated functions and biological phenomena associated with glycosphingolipids.

I MODIFICATION OF STRUCTURAL CHARACTERISTICS OF THE MEMBRANE MATRIX	(Abrahamsson et al., 1977; Karlsson, 1977a; 1982)
II EXPRESSION OF CELL-SURFACE MARKERS AND ANTIGENS ASSOCIATED WITH THE FOLLOWING PHENOMENA	
A. Immune recognition	
1. Blood group antigens	(McKibbin, 1978; Watkins, 1978; Hakomori, 1981b; Marcus et al., 1981)
2. Heterophile antigens	(Siddiqui and Hakomori, 1971; Higashi et al., 1977; Merrick et al., 1978)
3. Lymphoid subpopulation markers	(Stein-Douglas et al., 1976; Schwarting and Marcus, 1977; Stein et al., 1978; Kasai et al., 1980; Young et al., 1980)
4. Autoimmune antigens	(Fry et al., 1974; Hruby et al., 1977; Niemann et al., 1978; Feizi et al., 1979; Schwarting et al., 1979; Hirano et al., 1980)
B. Differentiation and cellular interactions	
1. Early embryogenesis	(Szulman, 1977; Stern et al., 1978; Willison and Stern, 1978; Solter and Knowles, 1978; Larraga and Edidin, 1979; Kapadia et al., 1981)
2. Intestinal epithelium differentiation	(Bouhours and Glickman, 1976; Glickman and Bouhours, 1976; Bouhours and Bouhours, 1981; Breimer et al., 1981a)
3. Blood cell differentiation	(Hakomori and Fukuda, 1980; Habu et al., 1980; Larson and Samuelsson, 1980; Saito et al., 1980; Schwarting and Summers, 1980; Momoi et al., 1982)
4. Cellular recognition	(Marchase, 1977; Obata et al., 1977; Mandel et al., 1980)

C. Cell growth and oncogenic transformation

1. Growth regulation (Hakomori, 1970; Gahmberg and Hakomori, 1975; Rosenfelder et al., 1979)

2. Oncogenic transformation (Hakomori et al., 1967; Häkkinen, 1970; Levine, 1976; Sundsmo and Hakomori, 1976; Hakomori et al., 1977; Rosenfelder et al., 1977; Breimer, 1980; Young et al., 1981a)

III ACTING AS RECEPTORS FOR DIFFERENT LIGANDS

A. Hormones (Lee et al., 1976; Beckner et al., 1981)

B. Toxins (Kato and Naiki, 1976; Ledley et al., 1977; Holmgren et al., 1980b; Kitamura et al., 1980; Critchley et al., 1981; Holmgren, 1981)

C. Drugs (Loh et al., 1978; Rösner et al., 1979; Karlsson, 1977b; 1982)

D. Immune mediators (Ankel et al., 1980; Miura et al., 1979; Liu et al., 1982)

E. Inorganic ions (Rahmann, 1980; Svennerholm, 1980; Karlsson, 1977a; 1982)

F. Viruses (Holmgren et al., 1980b; Markwell et al., 1981)

G. Bacteria (Källenius et al., 1980; Leffler and Svanborg-Edén, 1980)

Glycosphingolipids (or membrane bound glycoproteins) with blood group ABH activities have been postulated as differentiation markers in human embryos (Szulman, 1964; Watanabe and Hakomori, 1976; Szulman, 1977). In mouse embryos or teratocarcinoma cell lines the F9 antigen (Artzt et al., 1973; Muramatsu et al., 1979), the Forssman antigen (Stern et al., 1978; Willison and Stern, 1978), the blood group Ii antigens (Kapadia et al., 1981), the stage-specific embryonic antigen-1 or SSEA-1 (Solter and Knowles, 1978; Gooi et al., 1981; Hounsell et al., 1981) and the Ter C surface antigen (Larraga and Edidin, 1979) have been correspondingly described as developmental antigens. Polyclonal or monoclonal (Köhler and Milstein, 1975; Milstein, 1981) antisera were used for cellular localization of the antigens using immunofluorescence techniques and a remarkable stage-dependent change was observed. Thus, cell wall blood group ABH-antigens of human embryos (Szulman, 1964; Szulman, 1971) were detectable on all epithelia only at the earliest stages of development, the central nervous system, adrenals and liver being the exceptions. At the end of the first trimester, coincident with the morphologic and functional maturation of many organs (for example secretion of mucus from the gastrointestinal tract), these antigens began to wane. Only the epithelia of the integument, esophagus and lower urinary tract showed persistence of the antigens into and throughout extrauterine life. In contrast, water-soluble blood group antigens first appeared at 8 weeks ovulation age, when the mucus-secreting apparatus began to function, and then persisted throughout life. In the mouse early embryogenesis, blood group I antigen was readily detectable on undifferentiated cells (Kapadia et al., 1981), SSEA-1 was first detected on blastomers of 8-cell stage embryos (Solter and Knowles, 1978) and not until the blastocyst had been formed did Forssman (Willison and Stern, 1978) and i (Kapadia et al., 1981) antigens become visible. The trophectoderm later lost its activity of both SSEA-1 and Forssman antigens but as a conclusion no clear relation to cell lineage could be found for these surface markers (Solter and Knowles, 1978; Willison and Stern, 1978; Springer, 1980; Stinnakre et al., 1981). Intact or fragments of antibodies

directed against cell surface antigens have been shown capable of interfering with organogenesis and early differentiation (Szulman, 1975; Kemler et al., 1977), additionally suggesting a function for cell surface antigens in development.

Other differentiation systems as the differentiation from crypt to villus cells in the adult intestinal epithelium of rat was accompanied by an increase in glucosylceramide and hematoside (sialyllactosylceramide)* and also of 2-hydroxy fatty acids (Glickman and Bouhours, 1976; Bouhours and Glickman, 1976; Breimer et al., 1981a), but by a decrease in the amount of tri- and tetraglycosylceramides (Bouhours and Glickman, 1976; Breimer et al., 1981a). These changes, except for the increase of hydroxylated fatty acids, were, however, distinctly different from those observed in the small intestine during the perinatal period of the rat (Bouhours and Bouhours, 1981).

The perinatal shift of blood group i to I activity of human erythrocytes (Race and Sanger, 1975) has recently been explained by changes in the carbohydrate chains of both glycosphingolipids (Kościelak et al., 1979; Larson and Samuelsson, 1980; Hakomori and Fukuda, 1980) and glycoproteins (Fukuda et al., 1979; Fukuda et al., 1979; Zdebska et al., 1980). The i antigen of fetal cells is a repeating N-acetyllactosamine structure (Galβ1→4GlcNAcβ1→ →3Galβ1→4GlcNAc), the I antigen of adult cells is a branched structure (Galβ1→4GlcNAcβ1→6Gal) (Niemann et al., 1978; Feizi et al., 1979; Watanabe et al., 1979a). The branching process affects not only Ii antigens but also ABH antigens of these cells (Watanabe and Hakomori, 1976; Romans et al., 1980). In mouse and rat, T lymphocyte maturation has been associated with ganglio-tetraosylceramide (Schwarting and Summers, 1980; Habu et al., 1980; Momoi et al., 1982) and the ganglioside sialylgangliotetraosylceramide was markedly increased when mouse myeloid cells were induced to differentiate into macrophages (Saito et al., 1980).

The present thesis is the result of efforts to find a useful human source for studies on glycosphingolipids during cell differ-

* IUPAC-IUB Commission on Biochemical Nomenclature (1978) Biochem. J. 171, 21-35. This system is used throughout this thesis.

entiation. In the growing fetus, epithelial cells lining the intestines are continuously being discharged into the intestinal lumen forming a major part of what is known as meconium, the first stools of a newborn child. The final meconium masses have been produced during most of the fetal period and provided there is no degradation of glycosphingolipids in the intestinal lumen before birth, a careful analysis should then provide information on glycolipids from most of the fetal period (Paper I).

2. ON THE NATURE OF MECONIUM

Meconium, the first stool of the newborn infant, is a viscid, odourless, greenish-brown to black, sterile material being passed within 24 hours after partus by more than 90% of newborns. Meconium itself was so named by Aristotle, who thought, because of its likeness to material obtained from the poppy (mekonion) that it kept the fetus asleep in the uterus. Hippocrates postulated its presence in fetal intestine as evidence for the idea that the fetus sucks inside the womb. To date, no physiological function has been found for meconium although its constituents may give us some clues.

The precise origin of the components of human meconium has not been well defined although it is generally considered to be an accumulation of tissue debris consisting mainly of desquamated epithelial cells of the alimentary tract and integument, blood cells, lanugo hair, fatty material from vernix caseosa, amniotic fluid and various intestinal secretions (Patzelt, 1936; Harries, 1978). The discharge of small intestinal epithelial cells into the lumen starts already at the 7-8 weeks stage (Patzelt, 1936; Grand et al., 1976), at the time when the first villi are formed, and persists throughout life. The rate of cell migration from crypt to villus in utero was one-third to one-half of that found in mature human intestine (Grand et al., 1976) i.e. two to four days. Erythrocytes, leukocytes and swallowed epidermal cells are successively also accumulating in the (distal) small intestine (Patzelt, 1936) and subsequently in the colon. At this time, the fourth month of fetal

life, bile, pancreatic and intestinal secretions contribute essentially to the composition of meconium. Its final character thus results from ingested, secreted and desquamated material but also from active resorption taking place during intrauterine life. Thus, there is an early enzymatic differentiation into active proteases, glycosidases and lysosomal enzymes (Grand et al., 1976) as well as a development of transport processes (Grand et al., 1976) in the human fetal intestine. The quantitative or qualitative consequences of these gastrointestinal activities are not fully understood.

About 70-75% of the total wet weight of meconium is water (Harries, 1978; Buchanan and Rapoport, 1952). Non-polar lipids, predominantly cholesterol, squalene, bile and fatty acids, contribute around 10% of the total dry weight (Bernhard and Lindlar, 1956; Knauff and Adams, 1968), plasma proteins around 5% and carbohydrate-containing substances 70-80% of the dry mass (Knauff and Adams, 1968). The water soluble fraction contains 80% of the total carbohydrate content and more than 60% of the total dry weight (Fraser and Clamp, 1975).

Yosida in 1928 demonstrated for the first time blood group ABH activity in human meconium. This activity rapidly diminished after the first few days of life (Witebsky and Satoh, 1933; Okajima, 1940), parallel to the colonization of the gut. Blood group specific substances in meconium were later found to correspond to the blood type of the baby and its ability to secrete blood group activity in its secretions (Buchanan and Rapoport, 1951). The carbohydrate nature and structural specificity of blood group ABH and Lewis antigenic determinants were successively settled in the late 1950s and early 1960s (Table III; for reviews see Watkins, 1978; Hakomori, 1981b). Since then, glycoproteins in meconium have been shown to carry ABH, Le^a (Fraser and Clamp, 1975; Côté and Valet, 1976) and N activities (Springer et al., 1966) and glycosphingolipids to carry blood group ABH, Lewis and P specificity (Present thesis) and also MN activities (Côté, 1970). During the last few years glycoconjugates of human meconium have recieved an increasing attention from several laboratories (Present thesis;

Magnani et al., 1981; Herlant-Peers et al., 1981; Nilsson et al., 1981; Smith, 1982) and some novel structures and conceptions have also evolved.

Table III. Structures of the A,B,H,Le^a and Le^b determinants

Blood group Specificity	Carbohydrate structure
A	GalNAcα1→3Galβ1→3or4GlcNAc 2 ↑ Fucα1
B	Galα1→3Galβ1→3or4GlcNAc 2 ↑ Fucα1
H	Galβ1→3or4GlcNAc 2 ↑ Fucα1
Le^a	Galβ1→3GlcNAc 4 ↑ Fucα1
Le^b	Galβ1→3GlcNAc 2 4 ↑ ↑ Fucα1 Fucα1

3. GLYCOSPHINGOLIPID METHODOLOGY

The following paragraphs will be concerned mainly with techniques for small and medium-sized glycolipids (up to 20 sugars) as they are the best characterized (Hakomori and Kobata, 1974; Sweeley and Siddiqui, 1977; McKibbin, 1978; Hakomori, 1981b; Karlsson, 1982; Ledeen and Yu, 1982) and also those within the range of our own present methodology.

3.1 Preparation of glycosphingolipids. The preparation of a pure glycosphingolipid involves extraction, removal of other lipid or non-lipid contaminants and finally the removal of other glycosphingolipid species. Several different preparative methods (Karlsson et al., 1973; Hakomori and Watanabe, 1976; McKibbin, 1976; Slomiany et al., 1976; Sweeley and Siddiqui, 1977; Iwamori and Nagai, 1978; Svennerholm and Fredman, 1980; Momoi and Wiegandt, 1980; Breimer et al., 1981a) have been described based on the extraction of tissues with organic solvents, alkaline hydrolysis, precipitation or partition, column chromatography on silicic acids, magnesium silicate or DEAE-cellulose with or without prior acetylation (Handa, 1963). Through improved ion exchange chromatography on DEAE-Sepharose (Iwamori and Nagai, 1978; Nagai and Iwamori, 1980) or Spherosil-DEAE-Dextran (Fredman et al., 1980), gangliosides (acid glycosphingolipids), and with the use of porous homogenous silica gel, Iatrobeds (Ando et al., 1976; Nagai and Iwamori, 1980) gangliosides and non-acid glycosphingolipids have both been separated with great efficiency. High pressure liquid chromatography (HPLC) has been introduced for separation and quantification of derivatized analogues (McCluer and Jungalwala, 1976; Suzuki et al., 1977; Gross and McCluer, 1980) but recently also for preparative separation of native glycosphingolipids (Watanabe and Arao, 1981).

The purity of a glycolipid fraction is usually primarily checked by thin-layer chromatography. This technique has been considerably improved by the use of new stationary phases allowing better resolution and reproducability. The technique is potentiated by the use of different solvent systems, either in one or two dimensions (Ledeen and Yu, 1982), different staining reagents, either chemical (Stahl, 1967) or immunological (Magnani et al., 1981; Brockhaus et al., 1981) and by the combined use of derivatized and non-derivatized glycolipids (Papers II and III; Smith et al., 1975; Ångström et al., 1982b,c). However, the criteria of a pure glycolipid cannot be met with thin-layer chromatography alone but needs more advanced structural analyses.

3.2 Chemical characterization of glycosphingolipids. Glycosphingolipids have usually been analysed as pure or partially purified compounds, although novel techniques have permitted analyses even on mixtures (see below). The traditional way to elucidate the structure of a glycolipid is to degrade it and then analyse the products. Thus, constituent sugars and their molar ratios, fatty acids and long-chain bases are accessible to gas chromatography after acid degradation and suitable derivatizations (Sweeley and Walker, 1964; Yang and Hakomori, 1971; Sweeley and Siddiqui, 1977; Rickert and Sweeley, 1978; Torello et al., 1980). Sugar sequences and anomerity of the glycosidic linkages may be analysed by sequential degradation with exoglycosidases and a parallel identification of the liberated sugars and lipid products (McKibbin 1978; Kobata, 1979). The binding positions between sugars are usually established by acid degradation of the permethylated glycolipid and analysis of the partially methylated alditol acetates by gas chromatography (Stellner et al., 1973a,b; Stoffel and Hanfland, 1973). Complementary information on sugar sequence and binding positions may be obtained by partial acid hydrolysis, periodate oxidation and Smith degradation (McKibbin, 1978). Anomerity may also be determined using either NMR spectroscopy (see below) or the chromium trioxide method (Laine and Renkonen, 1975; Rauvala, 1976). Due to the recent progress in gas chromatographic techniques, mono- to hexasaccharides may now be successfully analysed (Nilsson and Zopf, 1982), potentiating the methods of chemical (MacDonald et al., 1980; Momoi and Wiegandt, 1980; Svensson, 1980) and enzymatical (Fukuda et al., 1978; Kobata, 1979) degradation of glycosphingolipids.

Our own efforts have focused on primary structural characterization of glycolipids without use of degradative methods. Mass spectrometry has taken a central position at this department since the days of the late Professors Einar and Stina Stenhagen. Starting with the analysis of fatty acids (see Waller, 1972), the technique has successfully been developed to microscale analyses of complex lipids and carbohydrates (Karlsson, 1978), The progress has not primarily been dependent on novel instrumental equipments

but rather on a careful selection of derivatives and conditions for sample handling, as discussed by Karlsson (1976; 1978). Thus, glycolipids were initially analysed as their acetyl and trimethylsilyl derivatives but permethylated and permethylated-$LiAlH_4$--reduced derivatives soon proved to be superior, giving increased volatility and stability with a minimal mass increase (Karlsson, 1973; Karlsson et al., 1974). Direct inlet mass spectrometry of these two derivatives of glycolipids provides information on number and sequence of sugars, type of sugars (pentose, deoxyhexose, hexose, N-acetylhexosamine, N-acetylneuraminic acid and N-glycoloylneuraminic acid), some sugar linkage positions plus the ceramide composition and is as yet applicable to glycolipids with up to 12 sugars in the carbohydrate chain (Breimer et al., 1981b). We have also analysed, by electron ionization, a 25-sugar glycolipid with sequence ions for 13 sugars from the non-reducing end and 9 sugars from the ceramide end (Breimer et al., 1983). The information may possibly increase by the combined use of high--field magnets and soft ionization techniques (for a review, see Burlingame et al., 1982).

Proton NMR spectroscopy offers a non-consuming non-degradative method for the determination of sugar anomerity in glycoconjugates (Falk et al., 1979a,b,c; Vliegenthart et al., 1980; Dabrowski et al., 1980). The use of permethylated and permethylated-reduced glycolipids, has enabled us to get complete information on anomerity of glycolipids with up to 10 sugars (Breimer et al., 1982a), the combination of two derivatives giving complementary information on specific sugar linkage positions. Underivatized glycolipids are also accessible for ^{1}H-NMR analysis using suitable solvents (Dabrowski et al., 1980; Falk et al., unpublished). Recently, ^{13}C-NMR has also been employed for the characterization of complex glycosphingolipids (Koerner et al., 1981; Sillerud et al., 1982). A drawback of these spectroscopic methods is, however, the present requirement for rather large amounts of substances.

Glycosphingolipids in mixtures are possible to analyse provided that the methods employed either yield information sufficiently specific for each glycolipid species or contain a built-in separative

step. Mass spectrometry and proton NMR spectroscopy of permethylated and permethylated-reduced glycolipids give such a characteristic fingerprint of single species as to allow analyses of mixtures (Falk et al., 1981; Ångström et al., 1982a,b,c). The NMR analyses may also be developed in two dimensions (Yamada et al., 1980; Levy and Craik, 1981) or on high field magnets (Vliegenthart et al., 1980; Levy and Craik, 1981). The mass spectrometer may be coupled to gas (Nilsson and Zopf, 1982) or liquid (McNeil et al., 1982) chromatography or even to another mass spectrometer; tandem mass spectrometry (McLafferty, 1981) improving the structural information. However, the fractionated evaporation in a direct inlet system and the use of data programs (Breimer et al., 1979) have already proved highly valuable for mass spectrometric analyses of glycosphingolipid mixtures (Falk et al., 1981; Breimer et al., 1982b; Ångström et al., 1982a,b,c).

3.3 Immunological characterization of glycosphingolipids. Glycolipids are antigenic, i.e. they can elicit an immune response, and they are known to react with several antisera and lectins of defined carbohydrate specificities. Most of the methods used for immunological characterization have been based on immuno-precipitation, complement-fixation, hemagglutination or inhibition of hemagglutination with glycolipids dispersed in aqueous solutions either singular or as liposomes with auxiliary lipids or as adsorbed onto cells (Marcus, 1976; McKibbin, 1978). Some of these techniques are rather insensitive and also favor reactions only with certain antibody classes. Solid phase radioimmunoassays, RIA (Young et al., 1979; Smolarsky, 1980; Brockhaus et al., 1981; Karol et al., 1981) and enzyme-linked immunosorbent assays, ELISA (Holmgren et al., 1980a; Sanai et al., 1981) have now been described for glycosphingolipids. These techniques are sensitive and measures the primary interaction between antigen and antibody without being dependent on secondary reactions such as agglutination or complement fixation. The use of well defined monoclonal antibodies, either natural or produced by the hybridoma technique (Köhler and Milstein, 1975) will probably potentiate this technique of immuno-

logical characterization. However, mixtures of glycosphingolipids cannot be satisfactorily analysed on solid phase immunoassays without previous separation. This problem has now been overcome as toxins and antibodies have been shown to bind directly to glycolipids separated on thin-layer plates (Magnani et al., 1980; Brockhaus et al., 1981; Karlsson and Larson, 1982; Hansson et al., submitted).

4. CURRENT WORK

Based on the histological composition and immunological reactivity of human meconium (see above) and the known richness of glycolipids, especially fucolipids, in intestinal epithelia (McKibbin, 1969), we assumed that meconium should be an interesting and readily available source for the study of cell-surface antigens of human fetal tissue. The elegant immunofluorescence studies of blood group ABH activities in human fetuses (Szulman, 1964; 1977) did not have a chemical counterpart and also the finding of blood group ABH- and MN-activities in a lipid extract of meconium was encouraging (Côté, 1970). However, the human gastrointestinal tract shows an early enzymatic differentiation and lysosomal enzymes (e.g. acid phosphatase, arylsulfatase, α- and β-glucosidase, β-galactosidase, β-glucuronidase and N-acetyl-β-glucosaminidase) were present in the fetal intestine (10 weeks) even before organized lysosomal structures could be seen with the electron microscope (13 weeks) (Antonowicz et al., 1974; Grand et al., 1976). Few enzymatic studies had been done on meconium itself (Antonowicz et al., 1975; 1977; 1978) but blood group activity of the first stools was known to diminish after birth (Witebsky and Satoh, 1933; Okajima, 1940) parallel to the colonization of the gut by enteric bacteria, producing blood group degrading enzymes (Hoskins and Boulding, 1976). Thus, provided there was no degradation of glycolipids in the intestinal lumen before birth information on cell-surface antigens from most of the fetal period should be available (Paper I). However, considering the fact that the

intestine elongates approximately 1000-fold from the 5th to the 40th week of gestation (Grand et al., 1976), one could conclude that cells discharged at an early embryogenetic period would only contribute a very minor part to the final meconium masses. Also, the exact cellular and temporal residence of certain glycolipid species would not be immediately revealed. Oncofetal antigens, i.e. fetal antigens cross-reacting with tumor-associated antigens (Coggin and Anderson, 1974; Coggin and Ambrose, 1979), should also be possible to isolate from this source, if such antigens exist among glycolipids (Watanabe and Hakomori, 1976; Hakomori and Young, 1978; Hakomori, 1981a).

Our total lipid extracts of meconium (weight around 5% of the total wet weight and 25% of the total dry weight) were dominated by cholesterol and other non-polar lipids (Paper I; Bernhard and Lindlar, 1956). The cholesterol fraction showed a large quantitative variation between single individuals and was probably mostly a skin product. Glycerophospholipids were practically absent and sphingomyelin was just a minor component, which is in correspondence with the finding of very low phosphorous levels in meconium (Buchanan and Rapoport, 1952). The total non-acid glycosphingolipid fractions ranged from 5 to 10% of the total lipid extracts and comprised about 50% of all sphingolipids present in meconium. Among sphingolipids free ceramide, monohexosylceramide (cerebroside) and sulfatide (cerebroside 3-sulfate) were the altogether dominating species (Paper III). The amount of blood group active non-acid fucolipids obtainable from one individual's meconium was very high, i.e. about twice that of autopsy material from adult whole small intestine and about 50 times that of 1 unit (450 ml) of fresh adult blood (Papers I and III). The pattern of glycosphingolipids from single individuals was shown to vary according to blood group and secretor status (Papers I and IV; Buchanan and Rapoport, 1951) and was probably derived mostly from epithelial cells of the fetal intestine. Individual variability was evident not only among the major blood group glycolipids of four to seven sugars but also among minor and more slow-moving glycolipids (8 sugars or more). Paper IV is an illustration to the potential use of glycosphingolipids of human meconium for a chemical blood group typing of

single living individuals with a comparable immunological typing of blood and saliva. This paper also defines the unique usefulness of mass spectrometric and NMR-spectroscopic techniques for a fingerprinting of glycolipids in a mixture. The immunological characterization of glycolipid mixtures was initially performed using liposomes in a hemagglutination-inhibition system (Dahlgren et al., 1981; Ångström et al., 1982b,c) but has now been considerably improved using the technique of binding antibodies directly to thin-layer chromatograms (Brockhaus et al., 1981; Karlsson and Larson, 1982; Hansson et al., submitted), see Fig. 3 as an example.

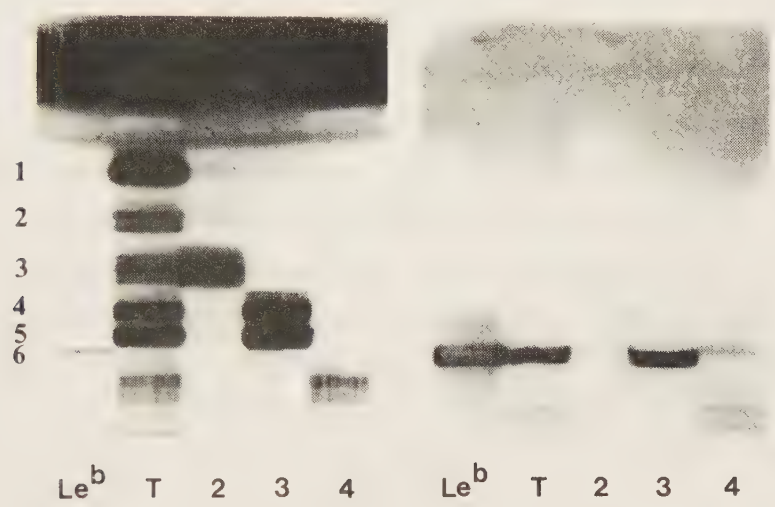

Fig. 3. Non-expected finding and identification of Le^b active glycosphingolipids in meconium of a phenotypically A Le(a+b-) non-secretor child. According to convention this phenotype should not express Le^b antigens (Race and Sanger, 1975). Two chromatograms were run identically in chloroform:methanol: water, 60:35:8 by vol., with 1 µg of a Le^b active hexaglycosylceramide (Le^b), 68 µg of total non-acid glycosphingolipids (T) and 9, 23 and 21 µg, respectively, of three consecutive subfractions (2, 3, 4) obtained from silicic acid column chromatography of the acetylated non-acid glycolipids. The left chromatogram was visualized with anisaldehyde reagent and the right chromatogram was autoradiographed (48 h) after labeling with a monoclonal anti-Le^b antibody (Brockhaus et al., 1981). Figures to the left indicate number of sugars in the carbohydrate chains. The two bands in the 4-5 sugar interval were identified as lactotetraosylceramide and a Le^a active pentaglycosylceramide using mass spectrometry, NMR-spectroscopy and hemagglutination-inhibition assays on subfraction 3.

The structural identities of all the major non-acid and acid glycosphingolipids of human meconium have been revealed by the present study (Papers II, III and IV), essentially using the techniques for structural characterization as described above. Patterns of co-presence of precursors and products were very characteristic for non-acid glycolipids of meconium (Papers III and IV). Lactotetraosylceramide, the possible precursor of type 1 chain (Galβ1→3 GlcNAc) ABH and Lewis active glycolipids (Table III; Watkins, 1978) was itself present in meconium together with its fucosylated products (Papers II and III). Likewise, all the intermediary structures up to fully glycosylated compounds were found in individuals of the corresponding phenotypes (Papers III and IV; Ångström et al., 1982b,c). The exact biosynthetic scheme of blood group active glycolipids has not yet been settled, however, and the patterns in meconium may also be a result of a fetal type of relative levels of different glycosyl-transferases, glycosyl-hydrolases and their substrates. One may note that although possibly present in plasma (Hanfland, 1978; Prohaska et al., 1978), meconium is still the only known human source of non-fucosylated or free lactotetraosylceramide (Paper II). Recently, Kabat and coworkers (1982) described a monoclonal IgMλ macroglobulin with specificity for lactotetraose in serum of a patient with a poorly differentiated bronchogenic carcinoma. The unusually favorable clinical course suggested that the lactotetraose structure recognized by the antibody could have been present in the patient's tumor as an oncofetal antigen (Kabat et al., 1982).

The presence of Le^a and Le^b active glycolipids in meconium, conclusively established in Paper III, is noteworthy. Firstly, Lewis antigens could not be demonstrated as cell wall constituents of human fetal intestine when using immunofluorescence techniques (Szulman and Marcus, 1973). Secondly, Lewis active glycolipids are usually not present on erythrocytes of cord blood (Race and Sanger, 1975; Larson and Samuelsson, 1980) but are secondarily acquired from the plasma after birth (Marcus and Cass, 1969; Race and Sanger, 1975; Rohr et al., 1980; Falk et al., 1981; Hanfland and Graham, 1981). The presence of Lewis active glycolipids in the

resting fetal gut, their absence on cord blood cells, and their successive appearance on erythrocytes after birth may thus suggest a route for synthesis and distribution associated with the entero-vascular changes upon oral feeding (Paper III). Similar to the glycolipids in other human gastrointestinal tissues (Falk et al., 1979d; Breimer et al., 1981c; 1982c) and plasma (Hanfland and Graham, 1981), the major ABH-determinants of glycolipids in meconium were thus built on the type 1 chain lactotetraosylceramide (Papers III and IV). This core structure was also found elongated by one or two disaccharides probably mostly of the type 2 (Galβ1→4 GlcNAc), and with the corresponding blood group ABH-determinants. Among those complex more slow-moving blood group glycolipids a novel series of trifucosyl compounds with an additional fucose at the inner hexosamine was also identified in meconium (Paper III; Ångström et al., 1982c; Larson, unpublished). The biosyntheses of these complex glycolipids are yet unknown and may involve different pools of transferases within a single cell. In addition to the blood group ABH-determinants on the lacto-series of glycolipids a minor blood group H active monofucosyl hexaglycosylceramide of the globo-series was isolated and characterized (Paper III). Due to small amounts available from a single individual, pooled meconia have been used for a preparative step, and a corresponding fraction is now being analysed. This structure represents a previously unknown linkage between the human ABH (Hakomori, 1981b) and P (Marcus et al., 1981) blood group systems, and analogous structures with A and B blood group determinants are likely to be found in meconium of children with the corresponding blood groups.

The gangliosides in meconium, and thus in fetal intestine, were much enriched compared to epithelial cells of adult intestines (Paper III; Holmgren et al., 1975; Falk et al., 1979d; Nilsson et al., 1981). The ganglioside sialyllactosylceramide was the dominating species in meconium (Paper III; Nilsson et al., 1981) as it is in adult intestine and in most other human extraneural tissues. The second most abundant ganglioside was identified as a NeuNAcα2→6neolactotetraosylceramide (Nilsson et al., 1981), earlier found in human erythrocytes (Watanabe et al., 1979b).

Minor known gangliosides were also identified and quantitatively determined (Nilsson et al., 1981). Recently, with the use of specific antisera or antibodies, also hitherto unknown gangliosides have been isolated from this source (Magnani et al., 1981; 1982; Smith, 1982). They represent the first sialylated type 1 chain glycolipids found, and one of them, a sialylated Le^a glycolipid, is the antigen of a tumor-specified monoclonal antibody (Magnani et al., 1981; 1982) obtained from immunization with a colon carcinoma cell line (Koprowski et al., 1979). This antigen is, however, also present in normal and tumor pancreas tissues (Hansson et al., submitted) from which we recently, using the same monoclonal antibody, isolated and characterized a ganglioside with an identical carbohydrate structure (Falk et al., submitted), see Fig. 4.

5. FUTURE WORK

Meconium has provided us with a readily available rich source of non-acid and acid glycosphingolipids. Although all the major glycolipids have been identified by now, numerous minor species probably remain to be discovered (unpublished observation). These may represent antigens of sparsely scattered cells or of cells from very early or short embryogenetic periods, and their structures may reveal novel combinations of sugars as already exemplified in a few cases (Paper III; Magnani et al., 1982; Smith, 1982). The easy access to large amounts of blood group type glycosphingolipids of the type 1 chain from meconium will be useful when collecting reference glycolipids for future work on carbohydrate conformation (Pascher and Sundell, 1977; Lemieux et al., 1980; Wright, 1980a,b) and biosynthesis (Prieels et al., 1981), for improvements of various biochemical techniques (Falk et al., unpublished; Karlsson et al., unpublished), for binding assays of biological ligands (Magnani et al., 1980; Brockhaus et al., 1981; Karlsson and Larson, 1982) and for production and characterization of immune sera and monoclonal antibodies (Brockhaus et al., 1981; Young et al., 1981b; Hansson et al., submitted). Monoclonal antibodies against glycolipids will be an interesting and perhaps the only

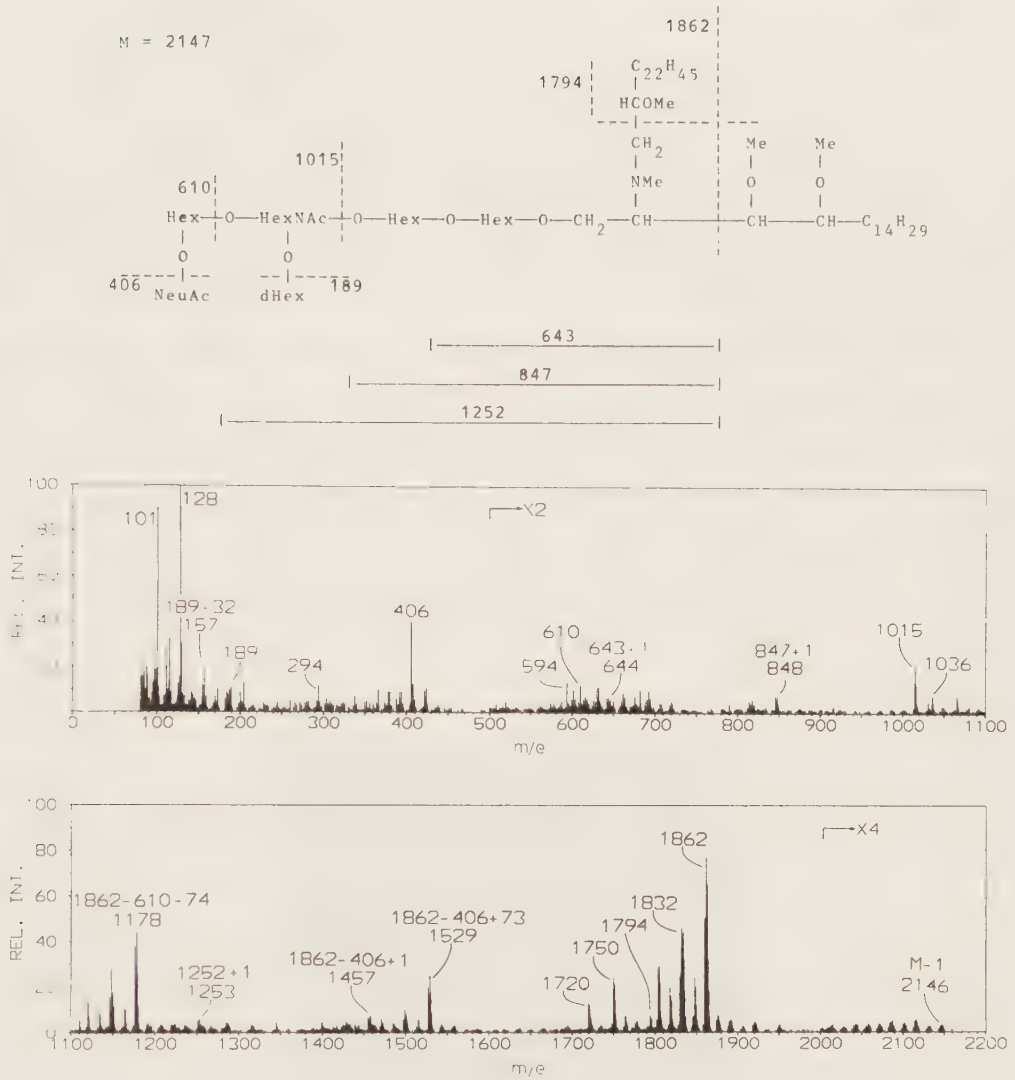

Fig. 4. Mass spectrum of the permethylated-reduced-trimethylsilylated fucoganglioside identified as the antigen (Magnani et al.,1982) of a tumor-specified monoclonal antibody reacting with gangliosides of meconium and colon carcinoma (Magnani et al.,1981; Karlsson and Larson,1982) and with normal and tumor pancreas tissues (Hansson et al.,submitted; Falk et al., submitted).The top formula illustrates the major fragmentation patterns of the heaviest ceramide species (trihydroxy 18:0 long-chain base and 2-hydroxy 24:0 fatty acid) but as shown in the spectrum also other ceramide species are present. The series of peaks at m/e 1036-1178 is specific for Lewis based glycolipids (Paper III).The sample was run in beam in the EI-mode on a ZAB-HF machine and the conditions of analysis were: electron energy, 62 eV; filament current, 200 μA; acceleration voltage 6 kV; and ion source temperature 220-280 °C. The spectrum was recorded at 270 °C.

efficient tool for the cellular and subcellular localization of these antigens in fetuses and adult tissues (Willison and Stern, 1978; Solter and Knowles, 1978; Milstein and Lennox, 1980; Kapadia et al., 1981; Sakakibara et al., 1981; Stinnakre et al., 1981). The individual patterns of blood group type glycosphingolipids in meconium are possible to correlate immunologically with blood, saliva or other products of the growing child (Paper IV) and may prove valuable for understanding the changing expression of blood group genotypes during differentiation and development (Szulman, 1977; Watkins, 1978). The finding of immunological cross-reactivities between antigens in meconium and tumor tissues (Magnani et al., 1981; 1982; Kabat et al., 1982) is interesting in the light of theories about dedifferentiation or derepression of the genome upon malignant transformation (Coggin and Andersson, 1974; Hakomori and Young, 1978; Hakomori, 1981a). Glycolipids from meconium will probably be useful, also in the future, for the isolation and characterization of such oncofetal antigens, at least for a screening procedure. The use of meconium for medical diagnoses has mainly been restricted to cystic fibrosis (Harries, 1978), but in the future the carbohydrate content may prove valuable for the screening also of other metabolic disorders (Larson, unpublished observation).

The biological functions of meconium, if any, are still obscure. Apart from being simply the result of various intestinal fetal activities, meconium might play an important role also in the perinatal period of life. The adhesion of uro-pathogenic enteric bacteria to cells by binding to specific sequences on glycolipids (Källenius et al., 1980; Leffler and Svanborg-Edén, 1980) is intriguing. This might suggest a protective role for glycolipids, and other glycoconjugates, in meconium. Thus, by a competitive binding of bacteria to meconium and not to intestinal epithelial cells, the massive infiltration and colonization of the intestines would be reduced and delayed until the protective factors of human colostrum and milk, i.e. bifidus factors, secretory IgA and plasma cells (Walker and Isselbacher, 1977) reach their destinations (L.-Å. Hansson, personal communication). Also, the carbohydrate

specificities of meconium and the high viscosity of this material might serve protective roles. Recently, the glycosphingolipid patterns of small intestine of rats and mice have been shown to undergo dramatic changes the first weeks after birth (Bouhours and Bouhours, 1981; Sato et al., 1982) as well as after the conventionalization of germ-free mice by feces of conventional mice (Umesaki et al., 1982). Feces of germ-free adult rats is relatively rich in glycolipids (about 2 mg per animal and day) and shows a pattern derived mainly from epithelial cells of small intestine (Fig. 5). This material may serve as a source for future studies on intestinal glycosphingolipids and their changes upon physiological and pathological stimuli and also perhaps on microbiological stimuli, such as the monocontamination with specific bacteria.

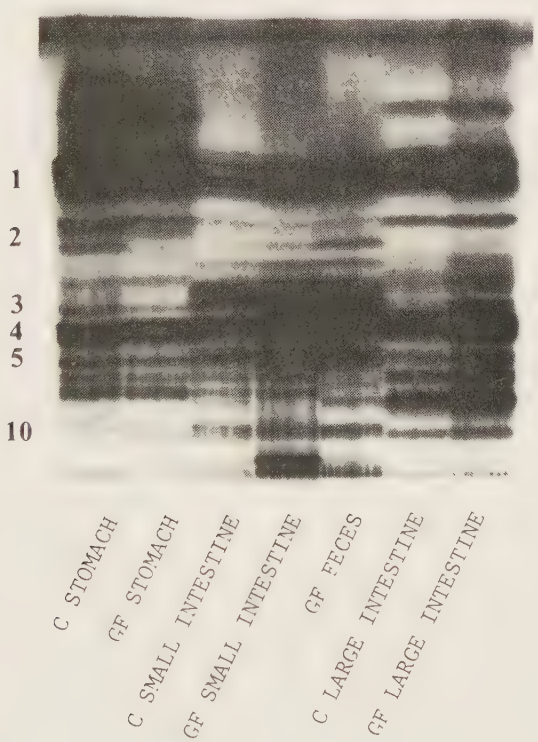

Fig. 5. Thin-layer chromatogram of total non-acid glycosphingolipids of stomach, small intestine, feces and large intestine of conventionalized (C) and germ-free (GF) rats (results in collaboration with B. Gustafsson and co-workers). The amount of glycolipid spotted on each lane corresponds to 40 mg dry weight of the corresponding tissues. Chloroform:methanol:water, 60:35:8 by vol., was used as solvent and anisaldehyde reagent for detection. Figures to the left indicate number of sugars of the glycolipids.

6. ACKNOWLEDGMENTS

My sincere thanks are due to my teachers in chemistry and biochemistry at all different levels throughout the years, my friends and colleagues at this department, my family and especially to Lena and Johanna for their personal support and inspiration.

7. REFERENCES

Abrahamsson, S., Dahlén, B., Löfgren, H., Paschér, I. and Sundell, S. (1977) in "Structure of Biological Membranes" (S. Abrahamsson and I. Pascher, eds.) pp. 1-23. Plenum Press, New York.

Ando, S., Isobe, M. and Nagai, Y. (1976) Biochim. Biophys. Acta 424, 98-105.

Ankel, H., Krishnamurti, C., Besançon, F., Stefanos, S. and Falcoff, E. (1980) Proc. Natl. Acad. Sci. USA 77, 2528-2532.

Antonowicz, I., Chang, S.K. and Grand, R.J. (1974) Gastroenterology 67, 51-58.

Antonowicz, I., Ishida, M.T. and Schwachman, H. (1975) Pediatrics 56, 782-787.

Antonowicz, I., Ishida, M.T. and Schwachman, H. (1978) Biol. Neonate 34, 225-230.

Antonowicz, I., Milunsky, A., Lebenthal, E. and Schwachman, H. (1977) Biol. Neonate 32, 280-289.

Artzt, K., Dubois, P., Bennett, D., Condamine, H., Babinet, C. and Jacob, F. (1973) Proc. Natl. Acad. Sci. USA 70, 2988-2992.

Beckner, S.K., Brady, R.O. and Fishman, P.H. (1981) Proc. Natl. Acad. Sci. USA 78, 4848-4852.

Bernhard, K. and Lindlar, F. (1956) Helv. Chim. Acta 39, 1443-1445.

Bouhours, D. and Bouhours, J.-F. (1981) Biochem. Biophys. Res. Commun. 99, 1384-1389.

Bouhours, J.-F. and Glickman, R.M. (1976) Biochim. Biophys. Acta 441, 123-133.

Breimer, M.E. (1980) Cancer Res. 40, 897-908.

Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H., Pimlott, W. and Samuelsson, B.E. (1979) Biomed. Mass Spectrom. 6, 231-241.

Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. (1981a) Exp. Cell Res. 135, 1-13.

Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H., Pimlott, W. and Samuelsson, B.E. (1981b) FEBS Lett. 124, 299-303.

Breimer, M.E., Karlsson, K.-A. and Samuelsson, B.E. (1981c) J. Biol. Chem. 256, 3810-3816.

Breimer, M.E., Falk, K.-E., Hansson, G.C. and Karlsson, K.-A. (1982a) J. Biol. Chem. 257, 50-59.

Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. (1982b) J. Biol. Chem. 257, 557-568.

Breimer, M.E., Karlsson, K.-A. and Samuelsson, B.E. (1982c) J. Biol. Chem. 257, 1079-1085.

Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Larson, G., Leffler, H., Pimlott, W., Samuelsson, B.E., Strömberg, N., Teneberg, S. and Thurin, J. (1983) J. MS and Ion Physics, in press.

Brockhaus, M., Magnani, J.L., Blaszczyk, M., Steplewski, Z., Koprowski, H., Karlsson, K.-A., Larson, G. and Ginsburg, V. (1981) J. Biol. Chem. 256, 13223-13225.

Buchanan, D.J. and Rapoport, S. (1951) Proc. Soc. Exp. Biol. Med. 77, 114-117.

Buchanan, D.J. and Rapoport, S. (1952) Pediatrics 9, 304-310.

Burlingame, A.L., Dell, A. and Russel, D.H. (1982) Anal. Chem. 54, 363R-409R.

Coggin, J.H. Jr. and Anderson, N.G. (1974) Adv. Cancer Res. 19, 105-165.

Coggin, J.H. and Ambrose, K.R. (1979) in "Methods in Cancer Research" (W.H. Fishman and H. Busch, eds.) Vol. 18, pp. 371-389. Academic Press, New York.
Côté, R.-H. (1970) in "Blood and Tissue Antigens" (D. Aminoff, ed.) pp. 249-264. Academic Press, New York.
Côté, R.H. and Valet, J.-P. (1976) Biochem. J. 153, 63-73.
Critchley, D.R., Magnani, J.L. and Fishman, P.H. (1981) J. Biol. Chem. 256, 8724-8731.
Dabrowski, J., Hanfland, P. and Egge, H. (1980) Biochemistry 19, 5652-5658.
Dahlgren, K.-E., Emilsson, P.M., Hansson, G.C. and Samuelsson, B.E. (1981) J. Immunol. Methods 44, 223-234.
Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E. (1979a) Arch. Biochem. Biophys. 192, 164-176.
Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E. (1979b) Arch. Biochem. Biophys. 192, 177-190.
Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E. (1979c) Arch. Biochem. Biophys. 192, 191-202.
Falk, K.-E., Karlsson, K.-A., Leffler, H. and Samuelsson, B.E. (1979d) FEBS Lett. 101, 273-276.
Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E. (1981) FEBS Lett. 124, 173-177.
Falk, K.-E., Karlsson, K.-A., Larson, G., Thurin, J., Blaszczyk, M., Steplewski, Z. and Koprowski, H. Biochem. Biophys. Res. Commun., submitted for publication.
Feizi, T., Childs, R.A., Watanabe, K. and Hakomori, S.-i. (1979) J. Exp. Med. 149, 975-980.
Fraser, D. and Clamp, J.R. (1975) Clin. Chim. Acta 59, 301-307.
Fredman, P., Nilsson, O., Tayot, J.-L. and Svennerholm, L. (1980) Biochim. Biophys. Acta 618, 42-52.
Fry, J.M., Weissbarth, S., Lehrer, G.M. and Bornstein, M.B. (1974) Science 183, 540-542.
Fukuda, M., Fukuda, M.N. and Hakomori, S.-i. (1979) J. Biol. Chem. 254, 3700-3703.
Fukuda, M.N., Fukuda, M. and Hakomori, S.-i. (1979) J. Biol. Chem. 254, 5458-5465.
Fukuda, M.N., Watanabe, K. and Hakomori, S.-i. (1978) J. Biol. Chem. 253, 6814-6819.
Gahmberg, C.G. and Hakomori, S.-i. (1973) J. Biol. Chem. 248, 4311-4317.
Gahmberg, C.G. and Hakomori, S.-i. (1975) J. Biol. Chem. 250, 2438-2446.
Glickman, R.M. and Bouhours, J.-F. (1976) Biochim. Biophys. Acta 424, 17-25.
Gooi, H.C., Feizi, T., Kapadia, A., Knowles, B.B., Solter, D. and Evans, M.J. (1981) Nature 292, 156-158.
Grand, R.J., Watkins, J.B. and Torti, F.M. (1976) Gastroenterology 70, 790-810.
Gross, S.K. and McCluer, R.H. (1980) Anal. Biochem. 102, 429-433.
Habu, S., Kasai, M., Nagai, Y., Tamaoki, N., Tada, T., Herzenberg, L.A. and Okumura, K. (1980) J. Immunol. 125, 2284-2288.
Hakomori, S.-i. (1970) Proc. Natl. Acad. Sci. USA 67, 1741-1747.
Hakomori, S.-i. (1981a) Ann. Rev. Biochem. 50, 733-764.
Hakomori, S.-i. (1981b) Seminars in Hematology 18, 39-62.

Hakomori, S.-i. and Fukuda, M. (1980) in "27th International Congress of Pure and Applied Chemistry" (A. Varmavuori, ed.) pp. 161-170. Pergamon Press, Oxford.
Hakomori, S.-i. and Kobata, A. (1974) in "The Antigens" (M. Sela, ed.) Vol. II, pp. 79-140. Academic Press, New York.
Hakomori, S.-i., Kościelak, J., Bloch, K.J. and Jeanloz, R.W. (1967) J. Immunol. 93, 31-38.
Hakomori, S.-i., Wang, S.-M. and Young, W.W. Jr. (1977) Proc. Natl. Acad. Sci. USA 74, 3023-3027.
Hakomori, S.-i. and Watanabe, K. (1976) in "Glycolipid Methodology" (L.A. Witting, ed.) pp. 13-47. Am. Oil Chem. Soc. Press, Champaign, Illinois.
Hakomori, S.-i. and Young, W.W. (1978) Scand. J. Immunol. 7 (suppl.), 97-117.
Handa, S. (1963) Japan. J. Exp. Med. 33, 347-360.
Hanfland, P. (1978) Eur. J. Biochem. 87, 161-170.
Hanfland, P. and Graham, H.A. (1981) Arch. Biochem. Biophys. 210, 383-395.
Hansson, G.C., Karlsson, K.-A., Larson, G., McKibbin, J.M., Blaszczyk, M., Herlyn, M., Steplewski, Z. and Koprowski, H. J. Biol. Chem., submitted for publication.
Harries, J.T. (1978) Br. Med. Bull. 34, 75-78.
Herlant-Peers, M.-C., Montreuil, J., Strecker, G., Dorland, L., Halbeek, H.v., Veldink, G.A. and Vliegenthart, J.F.G. (1981) Eur. J. Biochem. 117, 291-300.
Higashi, H., Naiki, M., Matsuo, S. and Okuchi, K. (1977) Biochem. Biophys. Res. Commun. 79, 388-395.
Hirano, T., Hashimoto, H., Shiokawa, Iwamori, M., Nagai, Y., Kasai, M., Ochiai, Y. and Okumura, K. (1980) J. Clin. Invest. 66, 1437-1440.
Holmgren, J. (1981) Nature 292, 413-417.
Holmgren, J., Elwing, H., Fredman, P. and Svennerholm, L. (1980a) Adv. Exp. Med. Biol. 125, 339-349.
Holmgren, J., Elwing, H., Fredman, P., Strannegård, Ö. and Svennerholm, L. (1980b) Adv. Exp. Med. Biol. 125, 453-470.
Holmgren, J., Lönnroth, I., Månsson, J.-E. and Svennerholm, L. (1975) Proc. Natl. Acad. Sci. USA 72, 2520-2524.
Hoskins, L.C. and Boulding, E.T. (1976) J. Clin. Invest. 57, 63-73.
Hounsell, E.F., Gooi, H.C. and Feizi, T. (1981) FEBS Lett. 131, 279-282.
Hruby, S., Alvord, E.C. Jr. and Seil, F.J. (1977) Science 195, 173-175.
Häkkinen, I. (1970) J. Natl. Cancer Inst. 44, 1183-1193.
Iwamori, M. and Nagai, Y. (1978) Biochim. Biophys. Acta 528, 257-267.
Kabat, E.A., Liao, J., Shyong, J. and Osserman, E.F. (1982) J. Immunol. 128, 540-544.
Kapadia, A., Feizi, T. and Evans, M.J. (1981) Exp. Cell Res. 131, 185-195.
Karlsson, K.-A. (1973) FEBS Lett. 32, 317-320.
Karlsson, K.-A. (1976) in "Glycolipid Methodology" (L.A. Witting, ed.) pp. 97-122. Am. Oil Chem Soc. Press. Champaign. Illinois.
Karlsson. K.-A. (1977a) in "Structure of Biological Membranes" (S. Abrahamsson and I. Pascher, eds.) pp. 245-274. Plenum Press, New York.

Karlsson, K.-A. (1977b) in "Abstracts 11th FEBS Meeting". A 4-2 600. Copenhagen.
Karlsson, K.-A. (1978) Prog. Chem. Fats other Lipids 16, 207-230.
Karlsson, K.-A. (1982) in "Biological Membranes" (D. Chapman, ed.) Vol. 4, pp. 1-75. Academic Press, London.
Karlsson, K.-A. and Larson, G. (1982) Adv. Exp. Med. Biol. 152, 15-23.
Karlsson, K.-A., Pascher, I., Pimlott, W. and Samuelsson, B.E. (1974) Biomed. Mass Spectrom. 1, 49-56.
Karlsson, K.-A., Samuelsson, B.E. and Steen, G.O. (1973) Biochim. Biophys. Acta 316, 317-335.
Karol, R.A., Kundu, S.K. and Marcus, D.M. (1981) Immunol. Commun. 10, 237-250.
Kasai, M., Iwamori, M., Nagai, Y., Okumura, K. and Tada, T. (1980) Eur. J. Immunol. 10, 175-180.
Kato, I. and Naiki, M. (1976) Infect. Immun. 13, 289-291.
Kemler, R., Babinet, C., Eisen, H. and Jacob, F. (1977) Proc. Natl. Acad. Sci. USA 74, 4449-4452.
Kitamura, M., Iwamori, M. and Nagai, Y. (1980) Biochim. Biophys. Acta 628, 328-335.
Knauff, R.E. and Adams, J.A. (1968) Proc. Soc. Exp. Biol. Med. 127, 801-804.
Kobata, A. (1977) in "The Glycoconjugates" (M.I. Horowitz and W. Pigman, eds.) Vol. I, pp. 423-440. Academic Press, New York.
Kobata, A. (1979) Anal. Biochem. 100, 1-14.
Koerner, T.A.W., Cary, L.W., Li, S.-C. and Li, Y.-T. (1981) Biochem. J. 195, 529-533.
Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D. and Fuhrer, P. (1979) Somat. Cell Genet. 5, 957-972.
Kościelak, J., Miller-Podraza, H., Krause, R. and Piasek, A. (1976) Eur. J. Biochem. 71, 9-18.
Kościelak, J., Zdebska, E., Wilczynska, Z., Miller-Podraza, H. and Dzierzkowa-Borodej, W. (1979) Eur. J. Biochem. 96, 331-337.
Källenius, G., Möllby, R., Svensson, S.B., Winberg, J., Lundblad, A., Svensson, S. and Cedergren, B. (1980) FEMS Microbiol. Lett. 7, 297-302.
Köhler, G. and Milstein, C. (1975) Nature 256, 495-497.
Laine, R.A. and Renkonen, O. (1975) J. Lipid Res. 16, 102-106.
Larraga, V. and Edidin, M. (1979) Proc. Natl. Acad. Sci. USA 76, 2912-2916.
Larson, G. and Samuelsson, B.E. (1980) J. Biochem. 88, 647-657.
Ledeen, R.W. and Yu, R.K. (1982) in "Methods in Enzymology" (V. Ginsburg, ed.) Vol. 83, pp. 139-191. Academic Press, New York.
Ledley, F.D., Lee, G., Kohn, L.D., Habig, W.H. and Hardegree, M.C. (1977) J. Biol. Chem. 252, 4049-4055.
Lee, G., Aloj, S.M., Brady, R.O. and Kohn, L.D. (1976) Biochem. Biophys. Res. Commun. 73, 370-377.
Leffler, H. and Svanborg-Edén, C. (1980) FEMS Microbiol. Lett. 8, 127-134.
Lemieux, R.U., Bock, K., Delbaere, T.-J., Koto, S. and Rao, V.S. (1980) Can. J. Chem. 58, 631-653.
Levine, P. (1976) Ann. N.Y. Acad. Sci. 277, 428-433.
Levy, G.C. and Craik, D.J. (1981) Science 214, 291-299.
Liu, D.Y., Petschek, K.D., Remold, H.G. and David, J.R. (1982) J. Biol. Chem. 257, 159-162.

Loh, H.H., Law, P.Y., Ostwald, T., Cho, T.M. and Way, E.L. (1978) Fed. Proc. 37, 147-152.

Macher, B.A. and Sweeley, C.C. (1978) in "Methods in Enzymology" (V. Ginsburg, ed.) Vol. 50, pp. 236-251. Academic Press, New York.

MacDonald, D.L., Patt, L.M. and Hakomori, S.-i. (1980) J. Lipid Res. 21, 642-645.

Magnani, J.L., Brockhaus, M., Smith, D.F., Ginsburg, V., Blaszczyk, M., Mitchell, K.F., Steplewski, Z. and Koprowski, H. (1981) Science 212, 55-56.

Magnani, J., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z. and Koprowski, H. (1982) Fed. Proc. 41, 898.

Magnani, J.L., Smith, D.F. and Ginsburg, V. (1980) Anal. Biochem. 109, 399-402.

Mandel, P., Dreyfus, H., Yusufi, A.N.K., Sarlième, L., Robert, J., Neskovic, N., Harth, S. and Rebel, G. (1980) Adv. Exp. Med. Biol. 125, 515-531.

Marchase, R.B. (1977) J. Cell Biol. 75, 237-257.

Marcus, D.M. (1976) in "Glycolipid Methodology" (L.A. Witting, ed.) pp. 233-245. Am. Oil Chem. Soc. Press, Champaign, Illinois.

Marcus, D.M. and Cass, L.E. (1969) Science 164, 553-555.

Marcus, D.M., Kundu, S.K. and Suzuki, A. (1981) Seminars in Hematology 18, 63-71.

Markwell, M.A.K., Svennerholm, L. and Paulson, J.C. (1981) Proc. Natl. Acad. Sci. USA 78, 5406-5410.

McCluer, R.H. and Jungalwala, F.B. (1976) Adv. Exp. Med. Biol. 68, 533-554.

McKibbin, J.M. (1969) Biochemistry 8, 679-685.

McKibbin, J.M. (1976) in "Glycolipid Methodology" (L.A. Witting, ed.) pp. 77-95. Am. Oil Chem. Soc. Press, Champaign, Illinois.

McKibbin, J.M. (1978) J. Lipid Res. 19, 131-147.

McLafferty, F.W. (1981) Science 214, 280-287.

McNeil, M., Darvill, A.G., Åman, P., Franzén, L.-E. and Albersheim, P. (1982) in "Methods in Enzymology" (V. Ginsburg, ed.) Vol. 83, pp. 3-46. Academic Press, New York.

Merrick, J.M., Zadarlik, K. and Milgrom, F. (1978) Int. Arch. Allergy Appl. Immunol. 57, 477-480.

Milstein, C. (1981) Proc. R. Soc. Lond. B. 211, 393-412.

Milstein, C. and Lennox, E. (1980) in "Current Topics in Developmental Biology" (A.A. Moscona and A. Monroy, eds.) Vol. 14, Part II, pp. 1-32. Academic Press, New York.

Miura, T., Handa, S. and Yamakawa, T. (1979) J. Biochem. 86, 773-776.

Momoi, T., Nakajima, K., Sakakibara, K. and Nagai, Y. (1982) J. Biochem. 91, 301-310.

Momoi, T. and Wiegandt, H. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1201-1210.

Muramatsu, T., Gachelin, G., Damonneville, M., Delarbre, C. and Jacob, F. (1979) Cell 18, 183-191.

Nagai, Y. and Iwamori, M. (1980) Mol. Cell. Biochem. 29, 81-90.

Niemann, H., Watanabe, K. and Hakomori, S.-i. (1978) Biochem. Biophys. Res. Commun. 81, 1286-1293.

Nilsson, B. and Zopf, D. (1982) in "Methods in Enzymology" (V. Ginsburg, ed.) Vol. 83, pp. 46-58. Academic Press, New York.

Nilsson, O., Månsson, J.-E., Tibblin, E. and Svennerholm, L. (1981) FEBS Lett. 133, 197-200.
Obata, K., Oide, M. and Handa, S. (1977) Nature 266, 369-371.
Okajima, S. (1940) Jap. J. Med. Sci. VII. Social Med. and Hyg. 3, 154-155.
Pascher, I. and Sundell, S. (1977) Chem. Phys. Lipids 20, 175-191.
Patzelt, V. (1936) in "Handbuch der Mikroskopischen Anatomie des Menschen" (W.v. Möllendorf, ed.) Vol. 5.3, pp. 1-448. Springer Verlag, Berlin.
Prieels, J.-P., Monnom, D., Dolmans, M., Beyer, T.A. and Hill, R.L. (1981) J. Biol. Chem. 256, 10456-10463.
Prohaska, R., Schenkel-Brunner, H. and Tuppy, H. (1978) Eur. J. Biochem. 84, 161-166.
Race, R.R. and Sanger, R. (1975) "Blood Groups in Man", 6th edn., Blackwell Scientific Publications, London.
Rahmann, H. (1980) Adv. Exp. Med. Biol. 125, 505-514.
Rauvala, H. (1976) J. Biol. Chem. 251, 7517-7520.
Rauvala, H. and Finne, J. (1979) FEBS Lett. 97, 1-8.
Rickert, S.J. and Sweeley, C.C. (1978) J. Chromatogr. 147, 317-326.
Rohr, J.E., Smith, D.F., Zopf, D.A. and Ginsburg, V. (1980) Arch. Biochem. Biophys. 199, 265-269.
Romans, D.G., Tilley, C.A. and Dorrington, K.J. (1980) J. Immunol. 124, 2807-2811.
Rosenfelder, G., van Eijk, R.V.W. and Mühlradt, P.F. (1979) Eur. J. Biochem. 97, 229-237.
Rosenfelder, G., Young, W.W. Jr. and Hakomori, S.-i. (1977) Cancer Res. 37, 1333-1339.
Rösner, H., Merz, G. and Rahmann, H. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 413-420.
Saito, M., Nojiri, H. and Yamada, M. (1980) Biochem. Biophys. Res. Commun. 97, 452-462.
Sakakibara, K., Momoi, T., Uchida, T. and Nagai, Y. (1981) Nature 293, 76-79.
Sanai, Y., Ogawa, J. and Nagai, Y. (1981) Japan. J. Exp. Med. 51, 309-316.
Sato, E., Uezato, T., Fujita, M. and Nishimura, K. (1982) J. Biochem. 91, 2013-2019.
Schwarting, G.A., Kundu, S.K. and Marcus, D.M. (1979) Blood 53, 186-192.
Schwarting, G.A. and Marcus, D.M. (1977) J. Immunol. 118, 1415-1419.
Schwarting, G.A. and Summers, A. (1980) J. Immunol. 124, 1691-1694.
Siddiqui, B. and Hakomori, S.-i. (1971) J. Biol. Chem. 246, 5766-5769.
Sillerud, L.O., Yu, R.K. and Schafer, D.E. (1982) Biochemistry 21, 1260-1271.
Singer, S.J. and Nicolson, G.L. (1972) Science 175, 720-731.
Slomiany, A., Galicki, N.I., Kojima, K., Banas-Gruszka, Z. and Slomiany, B.L. (1981) Biochim. Biophys. Acta 665, 88-91.
Slomiany, A., Slomiany, B.L. and Horowitz, M.I. (1976) in "Glycolipid Methodology" (L.A. Witting, ed.) pp. 49-75. Am. Oil Chem. Soc. Press, Champaign, Illinois.
Smith, D.F. (1982) Fed. Proc. 41, 1172.
Smith, E.L., McKibbin, J.M., Breimer, M.E., Karlsson, K.-A., Pascher, I. and Samuelsson, B.E. (1975) Biochim. Biophys. Acta 398, 84-91.

Smolarsky, M. (1980) J. Immunol. Methods 38, 85-93.
Solter, D. and Knowles, B.B. (1978) Proc. Natl. Acad. Sci. USA 75, 5565-5569.
Springer, T.A. (1980) in "Monoclonal Antibodies" (R.H. Kennett, T.J. McKearn and K.B. Bechtol, eds.) pp. 185-217. Plenum Press, New York.
Springer, G.F., Nagai, Y. and Tegtmeyer, H. (1966) Biochemistry 5, 3254-3272.
Stahl, E. (1967) in "Dünnschichtchromatographie" p. 817. Springer Verlag, Berlin.
Steck, T.L. and Dawson, G. (1974) J. Biol. Chem. 249, 2135-2142.
Stein, K.E., Schwarting, G.A. and Marcus, D.M. (1978) J. Immunol. 120, 676-679.
Stein-Douglas, K.E., Schwarting, G., Naiki, M. and Marcus, D.M. (1976) J. Exp. Med. 143, 822-832.
Stellner, K., Saito, H. and Hakomori, S.-i. (1973a) Arch. Biochem. Biophys. 155, 464-472.
Stellner, K., Watanabe, K. and Hakomori, S.-i. (1973b) Biochemistry 12, 656-661.
Stern, P.L., Willison, K.R., Lennox, E., Galfré, G., Milstein, C., Secher, D. and Ziegler, A. (1978) Cell 14, 775-783.
Stinnakre, M.G., Evans, M.J., Willison, K.R. and Stern, P.L. (1981) J. Embryol. Exp. Morph. 61, 117-131.
Stoffel, W. and Hanfland, P. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 21-31.
Sundsmo, J. and Hakomori, S.-i. (1976) Biochem. Biophys. Res. Commun. 68, 799-806.
Suzuki, A., Handa, S. and Yamakawa, T. (1977) J. Biochem. 82, 1185-1187.
Svennerholm, L. (1980) Adv. Exp. Med. Biol. 125, 533-544.
Svennerholm, L. and Fredman, P. (1980) Biochim. Biophys. Acta 617, 97-109.
Svensson, S. (1980) in "27th International Congress of Pure and Applied Chemistry" (A. Varmavuori, ed.) pp. 245-251. Pergamon Press, Oxford.
Sweeley, C.C. and Siddiqui, B. (1977) in "The Glycoconjugates" (M.I. Horowitz and W. Pigman, eds.) Vol. I, pp. 459-540. Academic Press, New York.
Sweeley, C.C. and Walker, B. (1964) Anal. Chem. 36, 1461-1466.
Szulman, A.E. (1964) J. Exp. Med. 119, 503-523.
Szulman, A.E. (1971) Human Path. 2, 575-585.
Szulman, A.E. (1975) Developmental Biol. 43, 101-108.
Szulman, A.E. (1977) in "Human Blood Groups" (J.F. Mohn, R.W. Plunkett, R.K. Cunningham and R.M. Lambert, eds.) pp. 426-436. Karger, Basel.
Szulman, A.E. and Marcus, D.M. (1973) Lab. Invest. 28, 565-574.
Torello, L.A., Yates, A.J. and Thompson, D.K. (1980) J. Chromatogr. 202, 195-209.
Umesaki, Y., Sakata, T. and Yajima, T. (1982) Biochem. Biophys. Res. Commun. 105, 439-443.
Vliegenthart, J.F.G., Halbeck, H.v. and Dorland, L. (1980) in "27th International Congress of Pure and Applied Chemistry" (A. Varmavuori, ed.) pp. 253-262. Pergamon Press, Oxford.
Walker, W.A. and Isselbacher, K.J. (1977) New Engl. J. Med. 297, 767-773.

Waller, G.R. (1972) in "Biochemical Applications of Mass Spectrometry" pp. 11-19. Wiley-Interscience, New York.
Watanabe, K. and Arao, Y. (1981) J. Lipid Res. 22, 1020-1024.
Watanabe, K. and Hakomori, S.-i. (1976) J. Exp. Med. 144, 644-654.
Watanabe, K., Hakomori, S.-i., Childs, R.A. and Feizi, T. (1979a) J. Biol. Chem. 254, 3221-3228.
Watanabe, K., Powell, M.E. and Hakomori, S.-i. (1979b) J. Biol. Chem. 254, 8223-8229.
Watkins, W.M. (1978) Proc. R. Soc. Lond. B. 202, 31-53.
Wilczynska, Z., Miller-Podraza, H. and Kościelak, J. (1980) FEBS Lett. 112, 277-279.
Willison, K.R. and Stern, P.L. (1978) Cell 14, 785-793.
Witebsky, E. and Satoh, T. (1933) Klin. Wchnschr. 12, 948-949.
Wright, C.S. (1980a) J. Mol. Biol. 139, 53-60.
Wright, C.S. (1980b) J. Mol. Biol. 140, 267-291.
Yang, H.-j. and Hakomori, S.-i. (1971) J. Biol. Chem. 246, 1192-1200.
Yamada, A., Dabrowski, J., Hanfland, P. and Egge, H. (1980) Biochim. Biophys. Acta 618, 473-479.
Yosida, K.I. (1928) Ges. Exp. Med. 63, 331-339.
Young, W.W. Jr., Hakomori, S.-i., Durdik, J.M. and Henney, C.S. (1980) J. Immunol. 124, 199-201.
Young, W.W. Jr., MacDonald, E.M.S., Nowinski, R.C. and Hakomori, S.-i. (1979) J. Exp. Med. 150, 1008-1019.
Young, W.W. Jr., Durdik, J.M., Urdal, D., Hakomori, S.-i. and Henney, C.S. (1981a) J. Immunol. 126, 1-6.
Young, W.W. Jr., Portoukalian, J. and Hakomori, S.-i. (1981b) J. Biol. Chem. 256, 10967-10972.
Zdebska, E., Magnuska, A., Kusnierz, G., Seyfried, H. and Kościelak, J. (1980) FEBS Lett. 120, 33-36.
Zopf, D.A., Ginsburg, V., Hallgren, P., Jonsson, A.-C., Lindberg, B.S. and Lundblad, A. (1979) Eur. J. Biochem. 93, 431-435.
Ångström, J., Briemer, M.E., Falk, K.-E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. (1982a) J. Biol. Chem. 257, 682-688.
Ångström, J., Falk, K.-E., Karlsson, K.-A. and Larson, G. (1982b) Biochim. Biophys. Acta 710, 428-436.
Ångström, J., Falk, K.-E., Karlsson, K.-A. and Larson, G. (1982c) Biochim. Biophys. Acta 712, 274-282.

ISBN 91-7222-531-9